Edis Travancic, Translational Sciences

Preclinical Development

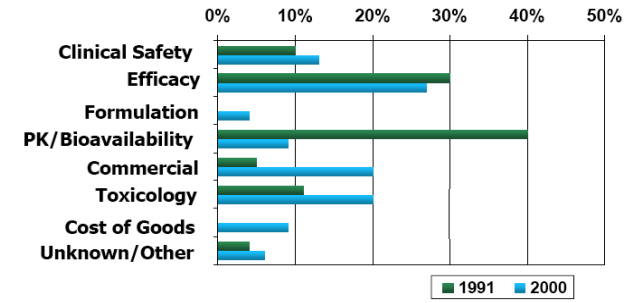
Abbreviations

A-B - Apical-to-basolateral
ADME - absorption, distribution, metabolism, excretion
AUC - Area under plasma concentration-time curve
B-A - Basolateral-to-apical
B.I.D. - *bis in die*, twice daily
BW - Body weight
BSA - Body surface area
C_{av} - Average concentration
C_p - Plasma concentration
C_{ss} - Stady-state concentration
C_T - Tissue concentration
C_u - Unbound concentration
CD - Candidate drug
CL - Clearance
CL_H - Hepatic clearance
CL_R - Renal clearance
C_{max} - Maximum plasma concentration
CL_{NR} - Non-renal clearance
CRO - Contract research organization
CSF - Cerebrospinal fluid
DMPK - Drug metabolism and Pharmacokinetics
E_H - Extraction ratio
(fraction lost during first-pass in liver)
EM - Extensive metabolizers
F_a - Fraction absorbed
f_b - Fraction bound
f_e - Fraction excreted
f_u - Fraction unbound
GFR - Glomerular filtration rate
GLP - Good laboratory practice
IP - Intraperitoneal
IV – Intravenous
K_p - Tissue-to-plasma partition coefficient
MDR - Multi-drug resistance
P_{app} - Apparent permeability
PD – Pharmacodynamics
P-gp - P-glycoprotein
PK - Pharmacokinetics
PM - Poor metabolizers
PO - Oral, *Per os*
POC - Proof-of-concept
QWBAR - Quantitative whole-body autoradiography
R&D - Research and development
SC - Subcutaneous
$t_{½}$ - Half-life
TK - Toxicokinetics
t_{max} - time point of maximum plasma concentration
τ - Dosing interval
V - Volume of distribution
wt - wild-type

Focus

- Drug metabolism and pharmacokinetics
- Oral administration
- Small molecules

Causes of attrition in drug development

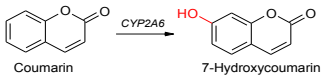


AstraZeneca, BMS, Lilly, Glaxo, J&J, Novartis, Pfizer, Pharmacia, Roche, Schering, SmithKline Beecham – over 500 programs surveyed.

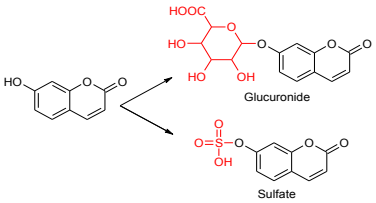
PMA/FDA Survey 1991, Pharmaceutical R&D Benchmarking Forum, General Metrics 2001

Drug metabolism

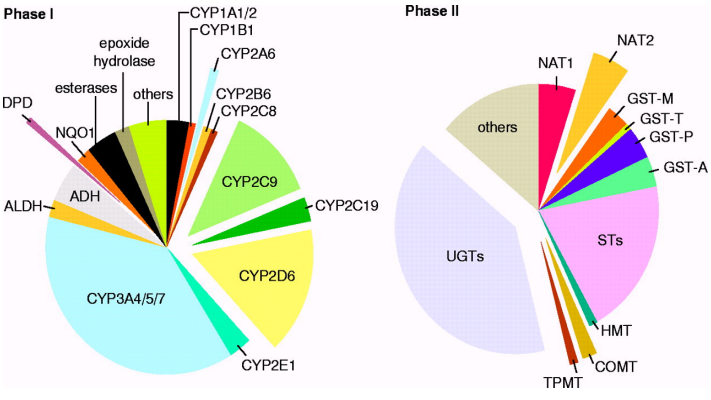
- Phase I reactions
  - Introduction of functional group (-OH, -COOH, -NH_2) by oxidation, reduction, hydrolysis, etc.
  - Preparation for Phase II metabolism or excretion



- Phase II reactions
  - Addition of endogenous component by glucuronide conjugation, sulfate conjugation, glutathione conjugation, methylation, acetylation
  - Increase in xenobiotic hydrophilicity resulting in excretion

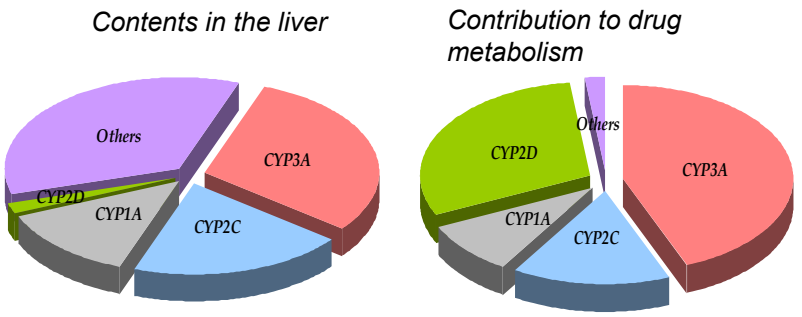


Drug metabolizing enzymes



Evans and Relling, Science 1999

Cytochrome P450



Shimada et al., J Pharmacol Exp Ther. 1994

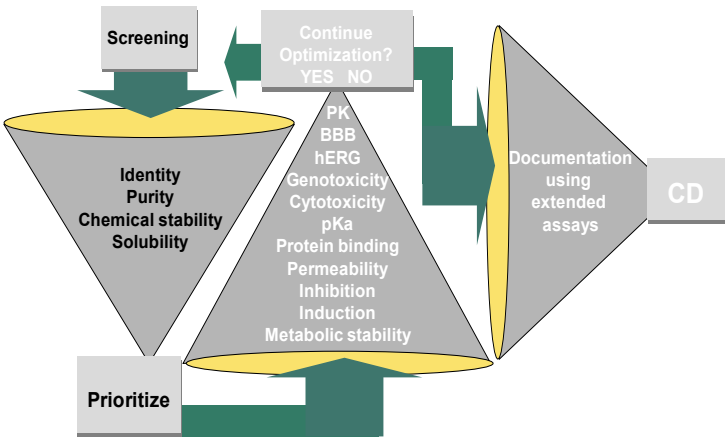
Genetic polymorphism in particular P450 isoform

- Poor metabolizers (PM) and extensive metabolizers (EM) of drugs metabolized by polymorph isoforms
- Criteria for therapeutically important genetic polymorphism:
  - An essential fraction of the given dose is metabolized by an polymorphic enzyme
  - A drug with a narrow therapeutic index
- CYP2D6
  - 5-10% Caucasians, and 0.9% Asians and Africans are PM
- CYP2C19
  - 2-5% Caucasians and 12-23% Asians are PM

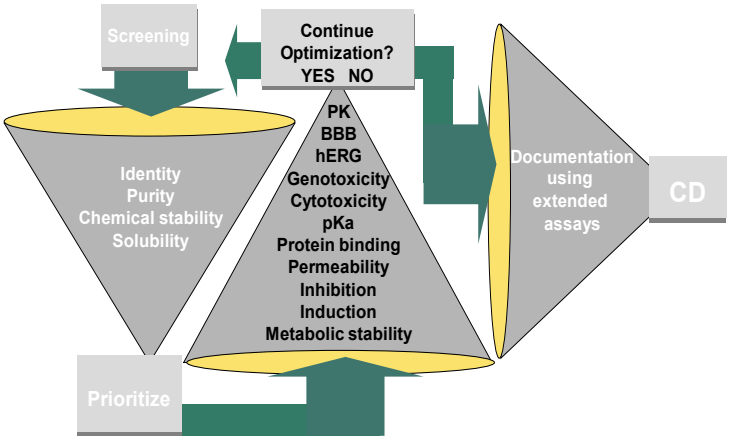
ADME in preclinical development

- Prediction of ADME in humans
- Pharmacology support
- Toxicology support

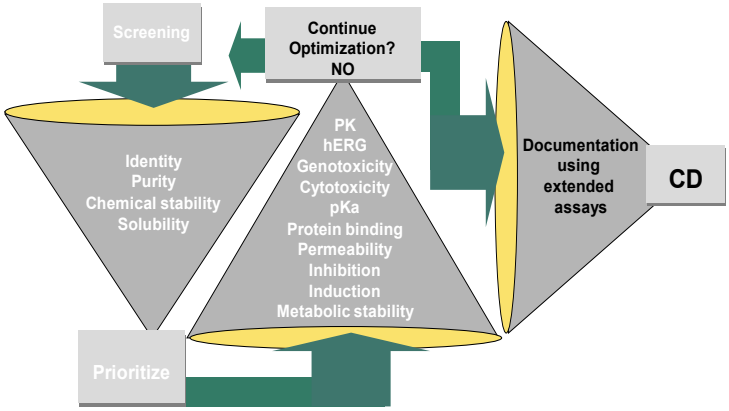
Screening for drug-like properties I



Screening for drug-like properties II



Screening for drug-like properties III



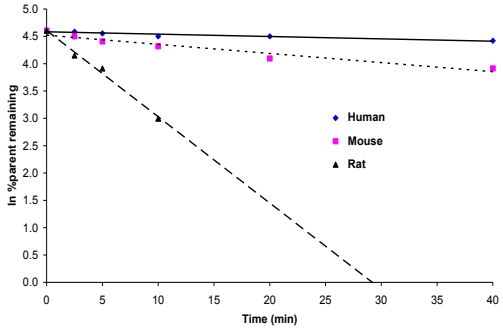
”Ideal” pharmacokinetic properties

- Complete absorption of an oral dose
- Elimination 50% via metabolism 50% via renal excretion
- Low clearance
- Low/moderate binding to plasma proteins
- No major metabolism via polymorphic enzymes
- No drug-drug interactions
- Linear pharmacokinetics
- No interaction with food

Metabolic stability studies

- Prediction of hepatic clearance and oral bioavailability (first-pass metabolism)
- Liver microsomes, hepatocytes, recombinant expressed individual CYP’s, liver slices, etc.

Metabolic stability studies



- Percent of parent compound remaining at each time point compared to control (0 min)
- Plot ln relative amount of parent compound remaining vs. time

→ *in vitro* $t_{1/2}$ → calculation (prediction) of CL_H and E_H

Metabolic stability studies

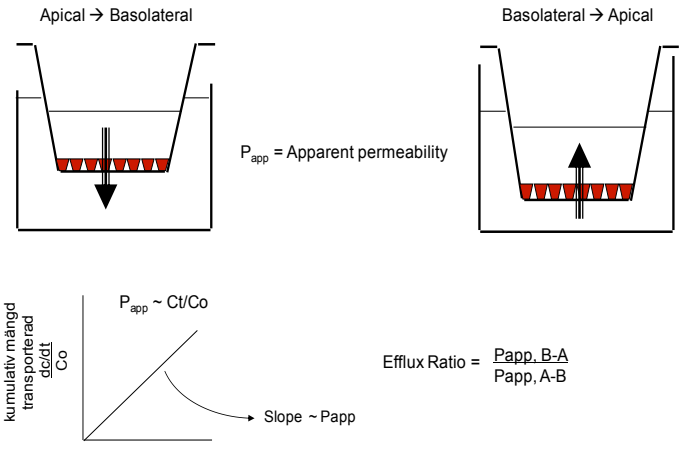
Interpretation of results

- Risk for high clearance
  - $E_H > 0.8$
- Intermediate compounds
  - $0.2 < E_H < 0.8$
- Low clearance compound
  - $E_H < 0.2$

Cell permeability studies

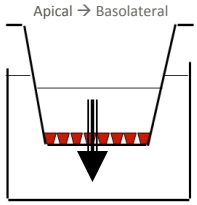
- Prediction of oral bioavailability (fraction absorbed in gastrointestinal tract) and distribution (e.g. CNS)
- Caco-2 cells (human colon cancer cell line)
- MDCK (Madin-Darby Canine Kidney) cells
  - Wild-type cells vs. MDR gene-transfected cells

Cell permeability studies

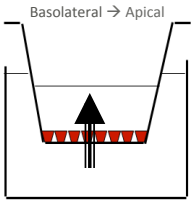


Cell permeability of BVT.2989

Cell type	Cell permeability P_{app} (µcm/s)	Efflux ratio
Caco-2	A-B: 30 B-A: 72	2.4
MDCK-MDR1	A-B: 6.5 B-A: 90	13.8
MDCK wt	A-B: 26 B-A: 71	2.7



P_{app} = Apparent permeability



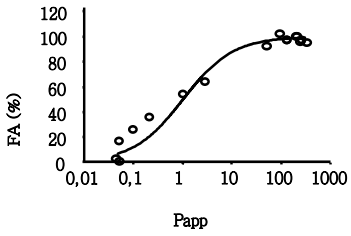
$$\text{Efflux Ratio} = \frac{P_{app,B-A}}{P_{app,A-B}}$$

Cell permeability studies

Interpretation of results

Low ↓ **Medium** ↓ **High**

1.5×10^{-6} cm/s 12×10^{-6} cm/s

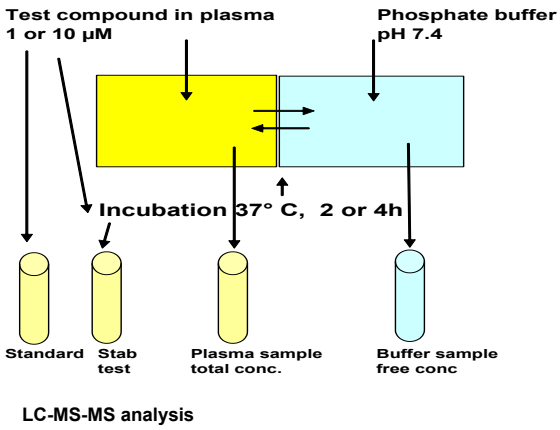


Plasma protein binding studies

- Determination of free fraction (i.e. fraction that is responsible for pharmacological and toxic effects) in plasma from humans as well as pharmacological and toxicological species
- *In vitro* methods such as equilibrium dialysis, ultrafiltration and ultracentrifugation

Plasma protein binding studies

Equilibrium dialysis



Plasma protein binding studies

Interpretation of results

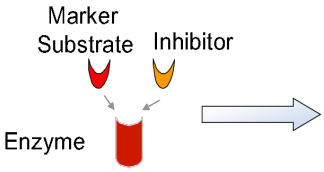
fb = bound fraction

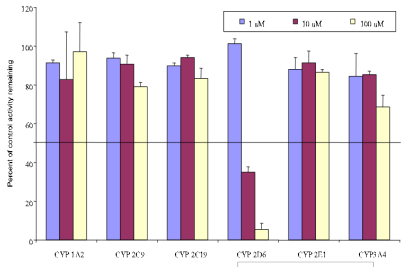
fb > 99 %	Predicted as very high
fb > 90 %	Predicted as high
fb 50 - 90 %	Predicted as moderate
fb < 50 %	Predicted as low
Stability > 80%	Stable
Recovery 80-120 %	Normal recovery

Cytochrome P450 inhibition studies

- Identify potent metabolic based CYP inhibitors and hence potential risk for drug-drug interaction(s)
- Recombinant expressed human CYPs or human liver microsomes
- 1A2, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4

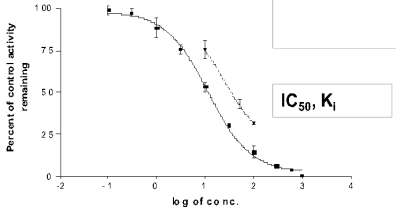
Cytochrome P450 inhibition studies





Marker reactions for CYP450s:

1A2 - chlorzoxazone 6-hydroxylation,
2C8 - paclitaxel 6α–hydroxylation,
2C9 - diclofenac 4'-hydroxylation,
2C19 - *S*-mephenytoin 4'-hydroxylation,
2D6 - dextromethorphan *O*-demethylation,
3A4 - testosterone 6β-hydroxylation,
3A4 - triazolam 1'-hydroxylation



Cytochrome P450 inhibition studies

Interpretation of results

Inhibitor conc. [μM]	Percent of inhibiton [%]	Predicted K_i [μM]	Risk for interaction *in vivo*
1	> 50	< 1	*Most likely*
10	> 50	> 1 < 10	*Possibly*
100	> 50	> 10 < 100	*Unlikely*
100	< 50	> 100	*None*

Bjornsson, T.D. et al., 2003.

Prediction of clinical relevance of competitive P450 inhibition

$[I]/K_i$	Prediction/Risk
$C_{max}/K_i > 1$	Likely / High risk
$1 > C_{max}/K_i > 0.1$	Possible / Medium risk
$0.1 > C_{max}/K_i$	Remote / Low risk

Tucker G.T. et al., 2001.

Metabolite characterization and quantitation

- *In vitro*
  - Liver microsomes, hepatocytes, recombinant expressed enzymes
- *In vivo*
  - Plasma, urine or bile samples from pharmacokinetic, pharmacology or toxicology studies
- Identification of pharmacologically active metabolites as well as toxic metabolites
- Guidance in driving chemistry towards compounds with better metabolic stability or non-toxic metabolites
- Guidance in choice of toxicological species

Preclinical pharmacokinetic studies

- Single-dose exposure in rodents (PO/SC/IP)
  - Support for planning of pharmacology studies (prediction of pharmacologically effective dose and dosing frequency)
- Single-dose PK in rodents (PO/IV)
  - Characterization of the plasma pharmacokinetic parameters e.g. oral bioavailability, clearance and volume of distribution
  - Urine sampling for determination of renal clearance

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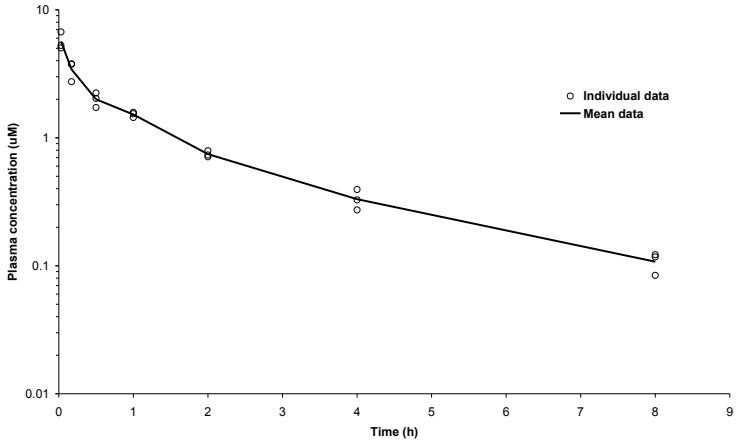
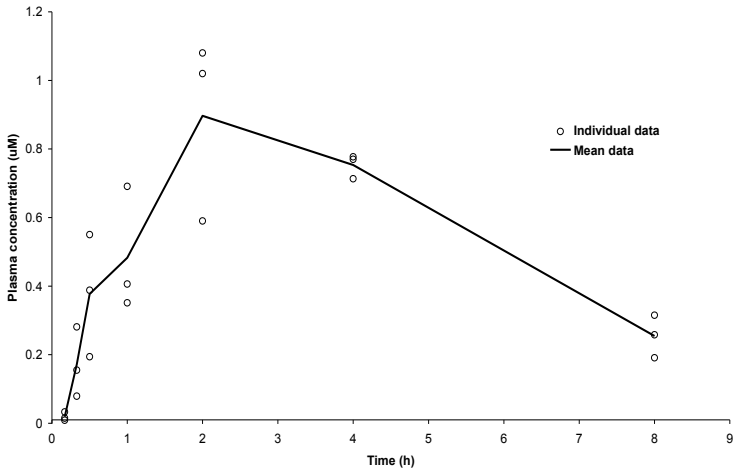
PK of BVT.A in male C57Bl mice

IV single-dose, 5 mg/kg



PK of BVT.A in male C57Bl mice

PO single-dose,10 mg/kg



PK of BVT.A in male C57Bl mice

Some PK parameters

Parameter	Method	Value
Oral bioavailability, F (%)	$(AUC_{oral} / AUC_{IV}) \bullet (Dose_{IV} / Dose_{oral})$	50
Total plasma clearance, CL (L/h·kg)	$Dose_{IV} / AUC_{IV}$	1.7
Fraction excreted unchanged in urine, f_e (%)	$A_e/Dose_{IV}$	0.08
Renal clearance, CL_R (L/h·kg)	$CL \bullet f_e$	0.001
Non-renal clearance, CL_{NR} (L/h·kg)	$CL - CL_R$	1.7

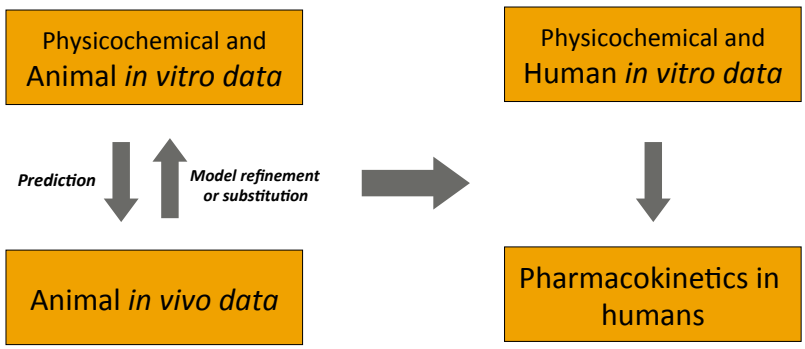
PK of BVT.A in male C57Bl mice

In vitro-in vivo comparison

Parameter	Actual value	Value predicted from *in vitro* data
Oral bioavailability (%)	50	37
Hepatic clearance, CL_H (L/h·kg)	1.7*	2.6
Renal clearance, CL_R (L/h·kg)	0.001	0.013 (i.e. GFR x f_u)

*Assumption: non-renal clearance = hepatic clearance

The way "we" work



Preclinical pharmacokinetic studies

- Repeat-dose exposure in connection with pharmacology studies
  - Support to interpretation of pharmacology results
  - Determination of clinically relevant plasma concentrations
- CNS distribution in rodents
  - Brain distribution in P-gp deficient mice (vs. wild-type)
  - Brain and CSF distribution in rats

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CNS distribution of BVT.2989 in P-gp deficient mice

10 mg/kg by subcutaneous infusion using osmotic minipumps during 24 h

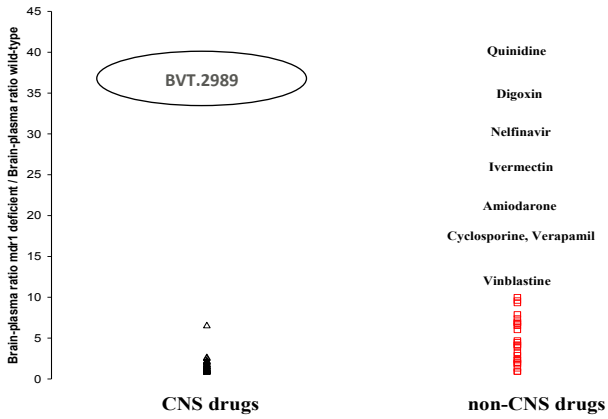
Wild-type and P-gp deficient CF-1 male mice

Plasma and brain sampling

	Wild-type	P-gp deficient
Plasma C_{ss} (µM)	0.35	0.46
Brain C_{ss} (nmol/g brain)	0.09	4.5
Brain C_{ss}/Plasma C_{ss}	**0.27**	**9.9**

37-fold difference in brain distribution between P-gp deficient and wild-type mice indicates limited CNS distribution of BVT.2989 due to P-gp dependent active transport

CNS distribution of various drugs in P-gp deficient mice



CNS distribution of BVT.2989 in rats

30 mg/kg by subcutaneous infusion using osmotic minipumps during 24 h

Sprague Dawley male rats

Plasma and CSF sampling

Plasma C_{ss} (µM)	0.59
Plasma $C_{ss,u}$ (µM)	0.44
CSF C_{ss} (µM)	0.03
CSF C_{ss}/Plasma $C_{ss,u}$	**0.07**

~15-fold difference between CSF and unbound plasma concentration indicates limited CNS distribution of BVT.2989

Preclinical pharmacokinetic studies

- Single-dose PK in non-rodents (PO/IV)
  - Characterization of the plasma pharmacokinetic parameters e.g. oral bioavailability, clearance and volume of distribution
  - Urine sampling for determination of renal clearance
  - PK-data from at least three species-more accurate prediction of PK in humans by means of allometric scaling
- Repeat-dose TK in rodents and non-rodents
  - Support to interpretation of toxicology results
  - Determination of marigin of exposure
  - Dose/time/gender dependent pharmacokinetics?

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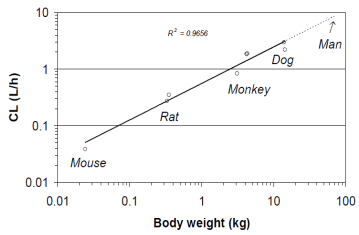
Prediction of PK in humans

Clearance (CL)	→	Animal data (allometric scaling) and/or human *in vitro* data
Volume of distribution (V)	→	Animal data (allometric scaling)
Oral bioavailability (F)	→	Human *in vitro* data, animal data and *in vitro-in vivo* comparison
Half-life ($t_{1/2}$)	→	Predicted volume of distribution and clearance

Allometric scaling

- The relationship between different physiological processes and body weight known for a long time
- Based on allometric relationships found for liver weight, blood flow, enzyme content, etc., common application of allometry in PK started in 1980s
- Body weight (BW) from several species is plotted against PK-parameter of interest



$$Y = a \cdot (BW)^b$$

$$\log Y = \log a + b \cdot \log$$

Allometric scaling

What we have learned so far

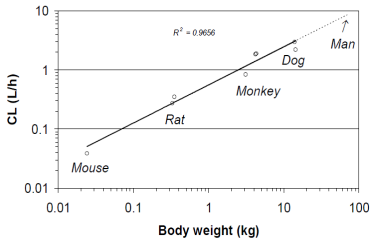
- Most useful for scaling of clearance and volume of distribution
- Inclusion of correction factors may improve accuracy of the prediction
  - MLP, brain weight, metabolic stability *in vitro,* plasma protein binding, bile flow, GFR, etc.
- As compared to clearance of metabolized drugs, tendency of more accurate predictions for volume of distribution in general as well as clearance of renally excreted drugs and protein therapeutics
- Choice of animal species included in allometry may influence accuracy of predictions
- In general, regarded to be a useful approach, however there are examples of poor predictions

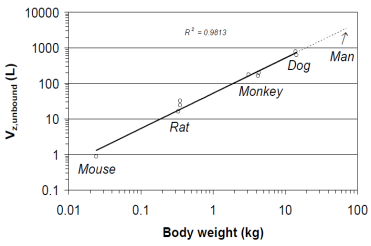
Allometric scaling

Prediction of PK in humans for BVT.3498

Allometric scaling of clearance and volume of distribution and prediction of AUC (=Dose/CL) and half-life (=ln2·V/CL)

Parameter	Predicted	Actual
AUC (µM·h)	5.2	5.3
Half-life (h)	7.0	5.8



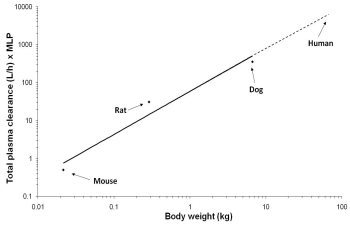


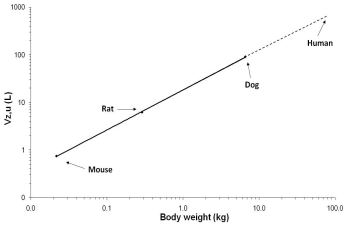
Allometric scaling

Prediction of PK in humans for BVT.28949

Allometric scaling of clearance and volume of distribution and prediction of AUC (=Dose/CL) and half-life (=ln2·V/CL)

Parameter	Predicted	Actual
AUC (µM·h)	0.15	0.11
Half-life (h)	2.5	1.6

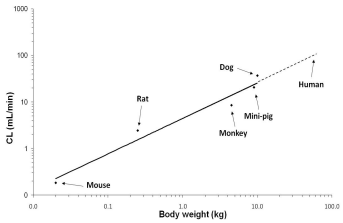




Allometric scaling

Susalimod

- Antireumatic agent in clinical development at Pharmacia & Upjohn during 1990s
- Extensivelly excreted in bile
  - In all species tested, 90% or more of administered dose was excreted in bile as unchanged drug
- Poor accuracy of prediction of clearance using allometric scaling
  - More than 20-fold overestimation of clearance in humans (125 ml/min vs. 5.2 ml/min)



Adapted from Påhlman et.al., Pharm Pharmacol Commun 1998

Identification of metabolizing enzymes

Enzyme kinetics in liver microsomes or hepatocytes
Determination of V_{max}, K_m and Cl_{int}

↓

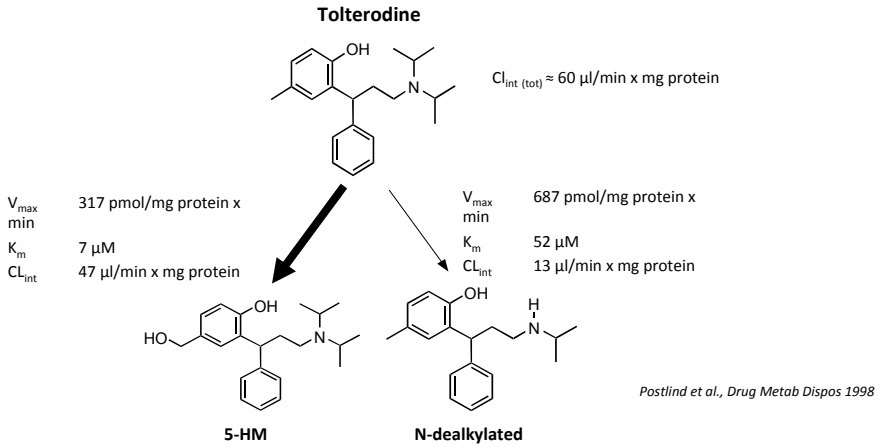
Formation of metabolite(s) in incubations with recombinantly expressed CYPs
Effect of specific chemical inhibitors against CYPs

↓

Characterization of CYP isoforms
involved in metabolic pathway(s)

Metabolism of tolterodine (Detrol®)

Enzyme kinetics in HLM



Postlind et al., Drug Metab Dispos 1998

Metabolism of tolterodine

Recombinantly expressed CYPs

Rate of formation (pmol/pmol P450 x min) for 5-HM and N-dealkylated tolterodine in recombinantly expressed P450 isoenzymes

CYP isoform	5-HM	N-dealkylated
1A1,1A2, 2C8	ND	ND
2C9	ND	0.25
2C19	ND	1.51
2D6	5.0	ND
3A4	ND	0.23

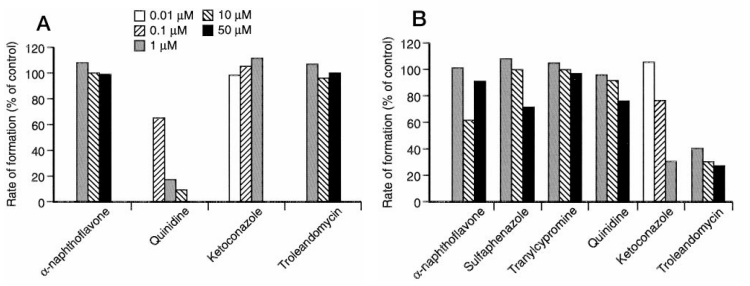
ND = Not detected

Postlind et al., Drug Metab Dispos 1998

Metabolism of tolterodine

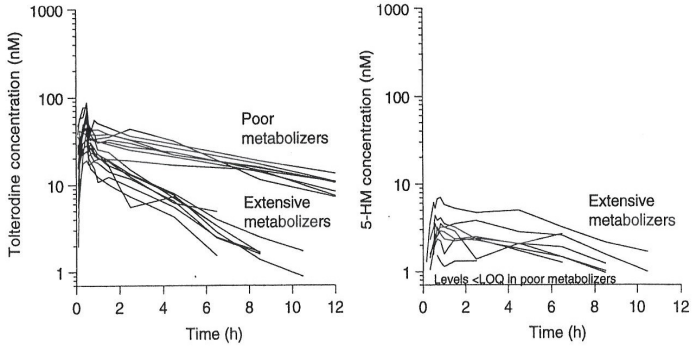
Chemical inhibitors

Effect of various cytochrome P450 inhibitors on the rate of formation of the 5-HM (A) and N-dealkylated tolterodine (B) in human liver microsomes



Postlind et al., Drug Metab Dispos 1998

PK-profiles of tolterodine and the 5-HM metabolite after intravenous infusion in humans



Brynne et al. Clin Pharmacol Ther 1998

Metabolism of tolterodine

Clinical significance

- Binding affinity to muscarinic receptors in urinary bladder
  - Tolterodine ~ 5-HM > N-dealkylated tolterodine
- Fraction unbound in human plasma
  - Tolterodine 3.7%
  - 5-HM 36%
  - N-dealkylated tolterodine 14%
- Unbound 5-HM is assumed to significantly contribute to the clinical efficacy of tolterodine in CYP2D6 EMs
- AUC_u for tolterodine in PMs ~ AUC_u for tolterodine + AUC_u for 5-HM in EMs

No significant difference in antimuscarinic effect between EMs and PMs